REMARKS

1. Election/Restriction

Reconsideration of the holding of lack of unity is respectfully sought in view of the present amendments and arguments.

With regard to point 1, while Stephan may disclose a composition comprising lysozyme and immunoglobulins, and the immunoglobulins may be inherently glycosylated as evidenced by Rudd, Stephan does not teach a composition comprising synthetically glycosylated immunoglobulins which (1) recognize surface antigens of Gram negative bacteria, and (2) are produced by being dissolved in a solution comprising disaccharide or monosaccharide, as required by amended claim 1.

Claim 1 further requires that as a result of such glycosylation, the bactericidal activity of the composition is increased.

With regard to point 3, we have added claim 32, reciting increased half life.

2. Claim Objections

The spelling error in claim 20 has been corrected.

3. Written Description Rejection (OA §4)

The Examiner questions whether there is an adequate description of the claimed genus of glycosylated immunoglobulins which are resistant to proteases and/or resistant to acidic conditions and/or lack the ability to fix complement.

At some length, the Examiner discusses the unpredictability of protein chemistry (OA pp. 5-6), and then presents, in very general terms, a summary of certain written description case law (p. 7). The examiner closes with conclusory statements as to applicants' disclosure.

In Example 2, it is reported that applicants made and

tested a composition as claimed. Hence, there is written description for at least one embodiment within the claim, and the issue is one of whether it is representative of the genus.

The specification teaches that immunoglobulins can be made resistant to proteases by glycosylating the Fc fragment. P9, L20-25.

Elsewhere in the rejection, the examiner states that Rudd discloses that "in the humoral immune system, all of the immunoglobulins are glycosylated". Such being the case, it is difficult to perceive how there could be a lack of written description for glycosylated immunoglobulins per se. Moreover, applicants disclose a general method for in vitro glycosylation of immunoglobulins, see P21, L33 to P22, L1. The Examiner has not explained why this method would not be generally applicable.

The Examiner questions whether applicants have adequately taught how to obtain glycosylated immunoglobulins with particular functionalities (protease resistance, see OA p. 4, acid resistance and lack of ability to fix complement, see OA p. 6). The reference to protease resistance is apparently prompted by claims 12-13, to acid resistance by claim 14, and to elimination of complement fixation by claim 15. However, the teachings of the disclosure provide written description for a genus of immunoglobulins with protease resistance, acid resistance and/or inability to fix complement.

Since the Fc fragment is similar for all immunoglobulins from a particular species (e.g. humans) and class (e.g., IgA, IgG, IgM), if a single IgG antibody which has been so glycosylated is resistant to protease, one would expect the same to be true of other IgG antibodies, too. (It is even more likely if the antibodies are of the same subclass, e.g., IgG3.)

Moreover, the fixing of complement is attributable to the Fc, see e.g., Benjamini and Leskowitz, Immunology:A Short Course 137 (Wiley-Liss 1991). Hence, if this is glycosylated,

the ability to bind complement can be lost. Because of the homology of Fc regions in immunoglobulins of the same species and class, if the glycosylation interferes with complement fixing in one such antibody, it would be expected to occur in essentially all antibodies from the same species and of the same class, see P9, L25-27. We have amended claim 15 to require that the ability of complement fixation is reduced. Losing the ability entirely is of course 100% reduction. But the point of the change is that to satisfy amended claim 15, the glycosylation need not abolish complement fixation, merely impair it. This is a laxer standard.

With regard to acid resistance, this is attributed by page 10, lines 26-28 to glycosylation. The Examiner has failed to adduce any evidence or reasoning which establish a prima facie basis for doubting that glycosylation could increase acid resistance.

The Examiner's attention is respectfully directed to the Written Description Training Materials, Example 13. This notes that "production of antibodies against a well-characterized antigen was conventional" and that "antibody technology is well developed and mature".

4. Definiteness Rejection (OA §5)

Claims 12-15 stand rejected as indefinite.

Claim 12 recited the phrase "wherein the glycosylated immunoglobulins are intact and/or resistant to proteases such as bacterial proteases and/or pancreatic proteases".

While the examiner did not question "intact and/or resistant", we have reduced this to simply "resistant". If an immunoglobulin is "resistant" to protease attack, it may remain intact, or it may be degraded but to a lesser degree than a non-resistant immunoglobulin. Whether it remains intact will depend on the choice of proteases, the concentration, and the exposure time. Some molecules may remain intact and others be degraded. Applicants decided that

the emphasis should be on that the glycosylated immunoglobulins are "resistant".

The term "bacterial proteases and/or pancreatic proteases" means exactly what it says. That is, the immunoglobulins are glycosylated in such manner as to render them resistant to bacterial proteases, pancreatic proteases, or both. There are, of course, proteases which are neither, e.g., insect proteases.

We acknowledge that the words "such as" are improper and they have been deleted.

Similar amendments have been made to claims 13 and 14.

With regard to claim 15, there are standard assays, well known in the art, for complement fixation (see the Indiana State "Complement Fixation Assay", copy attached, and Wikipedia, "Complement Fixation", copy attached). If the antibody binds to an antigen on the surface of a foreign cell, forming an antigen-antibody complex, the activated complement components attack the cell membrane, lysis and killing the cell. It is readily observable whether cell lysis occurs, and if the glycosylated immunoglobulins are unable to cause such lysis, then the antibody has lost the ability of complement fixation. It was long ago shown that F(ab)2 fragments have lost that ability, i.e., that the complement binding sites are in the Fc regions.

5. Features of the present invention

The present invention, teaches that Gram negative bacteria become accessible to lysozyme by exposing them to a mixture of lysozyme and immunoglobulins that have been synthetically glycosylated.

In Example 2 of the present application, two consecutive experiments involving two different strains of Gram negative bacteria is described. Example 2a is an experiment wherein the Gram negative bacteria strains are incubated with lysozyme and native immunoglobulins specific for one of the Gram

negative bacteria strains. Subsequent agar plate culturing resulted in bacterial colonies of both types of Gram negative bacteria. Example 2b is an experiment wherein the Gram negative bacteria strains are incubated with lysozyme and synthetically glycosylated immunoglobulins specific for one of the Gram negative bacteria strains. Subsequent agar plate culturing did not result in bacterial colonies of the type for which the synthetically immunoglobulins were specific. These experiments clearly demonstrate that the synthetically glycosylation of the immunoglobulins have a profound bacteriolytic effect on Gram negative bacteria.

One could speculate that the reason for this may be due to the nature of native glycosylation of immunoglobulins and other proteins within the cell. Glycosylation can be divided into two major types: N-linked glycosylation and O-linked glycosylation. In native N-linked glycosylation, the saccharides are attached by the enzyme oligosaccharyltransferase to asparagine residues occurring in the tripeptide sequence Asn-X-Ser, Asn-X-Thr or Asn-X-Cys, where X could be any amino acid except proline (Pro). This glycosylation process takes place in the endoplasmic reticulum. Native O-linked glycosylation is also catalyzed enzymatically and involves coupling of saccharides to the hydroxyl containing amino acids serine (Ser) and threonine (Thr). O-linked glycosylation takes place at a later stage of protein processing, probably within the Golgi apparatus. The number of native glycosylation sites of different proteins may vary to a great extent, and as a consequence of the sequence specific N-glycosylation process, it could be anticipated that the majority of immunoglobulins contain several potential glycosylation sites that are not natively glycosylated. In addition, proteins contain several other potential glycosylation sites that are normally not glycosylated *in vivo*, e.g. N-glycosylation on lysine (Lys) residues and on the N-terminal of the proteins, as well as O-glycosylation on

tyrosine (Tyr) residues.

The synthetic glycosylation of the immunoglobulins of the present invention may thus be regarded as a process, wherein these immunoglobulins are being additionally glycosylated, and this may be the reason for the profound enhanced properties of the immunoglobulins of the present invention towards bacteriolysis compared to native immunoglobulins. This effect may be due to an altered conformation of the bacterial cell wall upon attachment of the synthetically glycosylated immunoglobulins. This results in the accessibility of lysozyme to the underlying peptidoglycan layer and subsequent degradation of Gram negative bacteria.

Further advantages of the synthetic glycosylation include protection of the immunoglobulins against bacterial proteases. Additional glycosylations will hereby result in a larger fraction of immunoglobulins that will be resistant to bacterial proteases and the immunoglobulins will remain functional for a prolonged period of time after administration, as compared to native immunoglobulins.

6. Novelty and non-obviousness (OA §6)

Examiner states that claims 1-14 and 16-26 are anticipated by, or obvious over Stephan et al. (U.S. Patent 4,734,279) as evidenced by Rudd et al. (Science 2001) and Shu et al.(Nahrung 1998). The applicant respectfully disagrees.

Stephan et al. discloses a pharmaceutical composition comprising native immunoglobulins and lysozyme. In col. 4 lines 6 -12 is described how the lysozyme cleaves between N-acetylmuraminic acid and N-actylglucosamine constituting the peptidoglycan layer of the bacterial cell wall, whereby lysis of the bacterium occurs. The resulting cell fragments are captured by immunoglobulins whereby the fragments may be supplied to immune cells and thus eliminated.

Stephan et al. does not disclose synthetically glycosylated immunoglobulins directed towards antigens present

on the surface of Gram negative bacteria. Thus, the present invention is novel in view of U.S. Patent 4,734,279.

Shu et al. discloses that polysaccharide attachment, including monosaccharides such as mannose, to lysozyme is critical for excellent emulsifying properties.

The present invention relates to compositions comprising lysozyme, and in some embodiments lysozyme conjugated to monosaccharides, as well as synthetically glycosylated immunoglobulins. Combining elements of the invention of Stephan et al. with the disclosures of Shu et al. will thus not provide compositions with the features of the present invention.

Examiner further argues that all immunoglobulins of the humoral system are glycosylated according to Rudd et al., which results in that the native immunoglobulins of Stephan et al. implicitly are comparable to the immunoglobulins of the present invention. This is not true as demonstrated by Example 2 described above. As shown in Example 2 of the present invention, native immunoglobulins carrying the native glycosylations have no bacteriolytic effect on Gram negative bacteria (Example 2a), whereas the synthetically glycosylated immunoglobulins of the present invention have a profound enhanced effect towards bacteriolysis of Gram negative bacteria (Example 2b). Furthermore, the fact that all native humoral immunoglobulins are glycosylated according to Rudd et al. further adds to the surprising properties of the present invention. The skilled artisan would not consider adding a feature to a composition that apparently already is present in the composition.

In conclusion, the present invention is both novel and non-obvious in light of the prior art due to the synthetic glycosylation of the immunoglobulins, thereby conferring new and improved features to the combined effect of lysozyme and immunoglobulins already known in the art. The present invention solves a problem, not already solved by Stephan et

al. in regard to both prolonged half-life of the glycosylated immunoglobulins (cp. claim 32) and effectiveness against Gram negative(cp. claims 3-5) and other bacteria.

Respectfully submitted,

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Enclosures
-1) Benjamini, Immunology: A Short Course (title page and page 137)
-2) "Complement fixation assay"
www1.indstate.edu/theme/PSP/labtests/complementfix.htm
-3) Wikipedia, "Complement fixation test"
www.en.wikipedia.org/wiki/complement_fixation_test

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